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A New Chromosome Number for the Genus *Medicago*[1]

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ABSTRACT

Seventy-two accessions of *Medicago scutellata* (L.) Mill. and 37 accessions of *M. rugosa* Desr. were screened to determine if diploid forms of these species exist and to observe the morphological variation within the two species. Diploid plants are desirable for crossing with both the diploid and tetraploid forms of *M. sativa* L. (alfalfa). The somatic chromosome number of 2n=30 was observed in all accessions of *M. scutellata* and *M. rugosa*, and no diploid forms were found. Both species contain satellited chromosomes (SAT-chromosomes), *M. scutellata* has two pairs of SAT-chromosomes and *M. rugosa* has one pair. The satellites are large and they are located a relatively long distance from the main body of the chromosome, possibly leading to the erroneous chromosome counts of 2n=32 recorded previously. Meiosis was regular in the microspore mother cells of both species with 15 bivalents forming at metaphase I. On the basis of intraspecific variation, plants of *M. scutellata* could be separated into three morphological groups: 1) nonvigorous plants that flower approximately 1 month after planting and have one flower per inflorescence, 2) vigorous plants that flower 2 months after planting and have three flowers per inflorescence, and 3) an intermediate group. The way in which these 2n=30 species may have evolved and their potential value in a breeding program are suggested.

***Additional index words:* *Medicago scutellata* (L.) Mill., *M. rugosa* Desr., Satellited chromosomes.**

A MAJOR portion of the cytogenetic research conducted on the genus *Medicago* L. has been directed towards the perennial species, and little attention has been given to the annual species. Some of the annual species may be a valuable source of germplasm for the improvement of the agriculturally important *M. sativa* L. (alfalfa) (Barnes et al., 1977).

It is generally agreed that the basic chromosome numbers for the genus *Medicago* are x = 7 and x = 8. Most species are 2n = 2x = 16, 2n = 4x = 32, or 2n = 6x = 48; however, five species [*M. constricta* Dur., *M. murex* Willd., *M. polymorpha* L., *M. praecox* DC., and *M. rigidula* (L.) All.] are 2n = 2x = 14 (Lesins and Lesins, 1979).

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Two annual species, *M. rugosa* Desr. and *M. scutellata* (L.) Mill, are of particular interest to alfalfa breeders. Barnes and Ratcliffe (1969) and Shade et al. (1975 and 1979) reported that both of these species are resistant to the potato leafhopper [*Empoasco fabae* (Harris)] and the alfalfa weevil [*Hypera postica* (Gyllenhal)] due to the glandular hairs on the stems and leaves. In addition, both species have been reported to be the only annuals that are tetraploids with 2n=32 (Ghimpu, 1930; Fryer, 1930; Senn, 1938; Heyn, 1956 and 1963; Clement, 1962; Simon and Simon, 1965; and Lesins and Lesins, 1979). Thus, hybridization between these two annual species and *M. sativa* may be possible because of their common ploidy level. However, crosses between species with unequal ploidy levels have sometimes been successful due to the production of unreduced gametes (Bingham and Saunders, 1974). The only successful hybridization between either of these annual species and *M. sativa* was reported by Sangduen et al. (1982), who recovered one plant from the cross *M. sativa* × *M. scutellata*. However, this plant was a chimera and sterile.

Heyn's (1963) description of *M. scutellata* states that ". . .intraspecific variation is mainly confined to the size of vegetative and reproductive organs, the range of variation of the size is often very wide. This leads us to assume that *M. scutellata* might comprise diploid forms." If diploid *M. scutellata* plants exist, they may be more compatible with *M. sativa* than tetraploid plants.

The purpose of this study was to: 1) observe intraspecific morphological variation within *M. scutellata* and *M. rugosa*, 2) determine if diploid forms exist, and 3) determine if there are any meiotic irregularities which may effect their hybridization with *M. sativa*.

MATERIALS AND METHODS

Seventy-two accessions of *M. scutellata* and 37 accessions of *M. rugosa* were grown under greenhouse conditions at Beltsville, Md. Twenty-seven and 30 accessions of *M. scutellata* and *M. rugosa*, respectively, were graciously provided to use from Lesins' *Medicago* collection by Dr. P. Seymour, Devonian Botanic Garden, Univ. of Alberta, Edmonton,

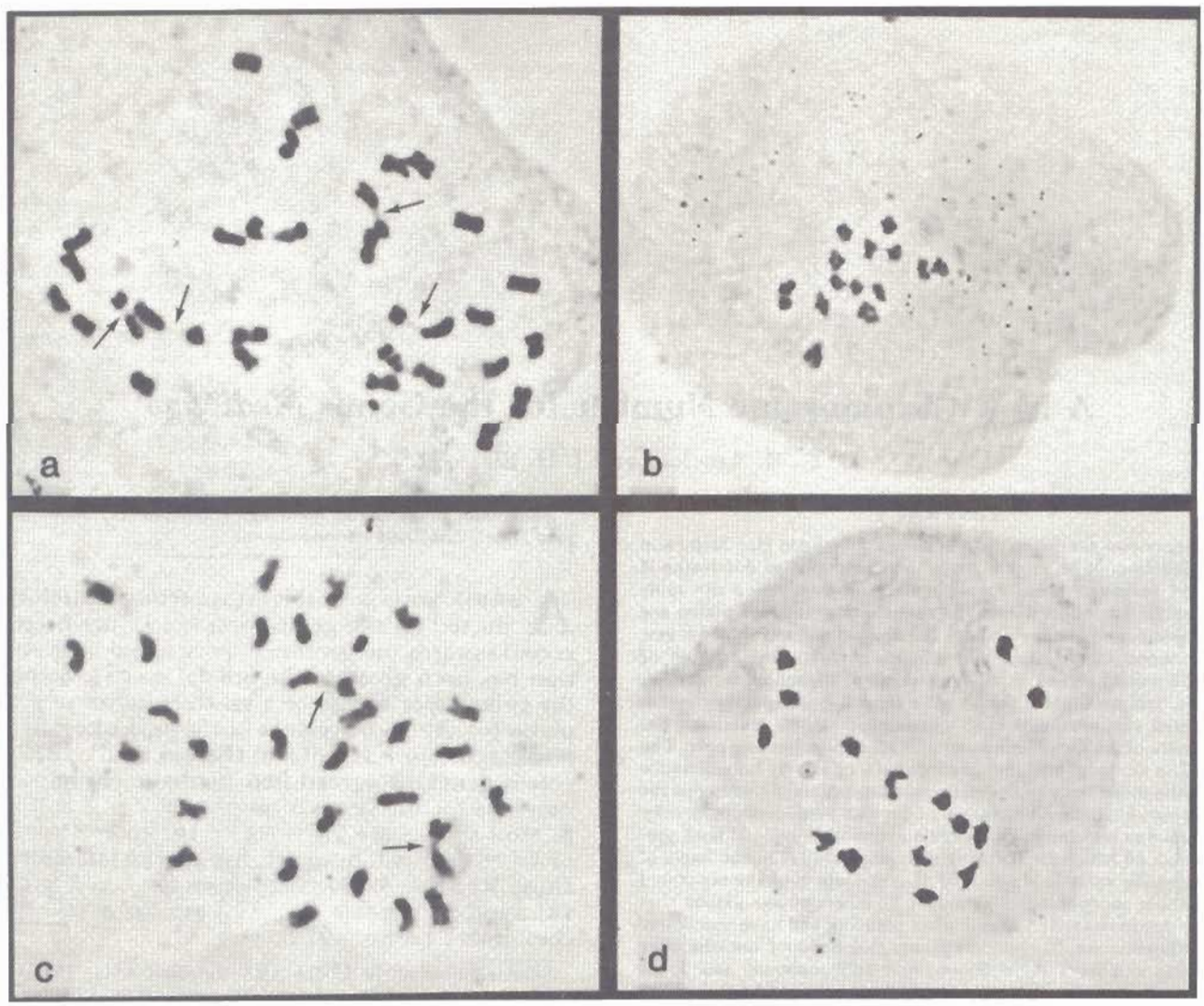

Fig. 1. Photomicrographs of the somatic and meiotic chromosomes of *Medicago scutellata* and *M. rugosa*. a) Somatic chromosomes of *M. scutellata* with 4 SAT-chromosomes (arrows), 2n=30, X1575; b) Metaphase I of *M. scutellata* showing 15_{II}, X1300; c) Somatic chromosomes of *M. rugosa* with two SAT-chromosomes (arrows), 2n=30, X1575; d) Metaphase I of *M. rugosa* showing 15_{II}, X1375.

Alberta, Canada. The remaining accessions were obtained through the U. S. Plant Inventory (PI) System. Five plants of each accession were grown in separate pots and the morphological and cytological data were collected from these plants.

Several root tips from each plant were collected, placed into a saturated solution of paradichlorobenzene at room temperature for 4 h, transferred to Carnoy's fluid (6:3:1, ethanol: chloroform: acetic acid), and stored in a refrigerator until cytological observations could be made. The root tips were hydrolyzed for 20 min in 1*N* HCl at 60°C, placed in Feulgen's stain for up to 24 h, and squashed in acetocarmine. Four root tips per plant (20 per accession) were squashed on a slide. Chromosome counts were made from slides which yielded 5 to 10 countable cells.

Meiotic observations were made on microspore mother cells (MMC's) from five accessions of each species. The flower buds were fixed in Carnoy's fluid for at least 24 h, the anthers were dissected, and the MMC's were squashed in acetocarmine. All stages of meiosis were observed.

RESULTS AND DISCUSSION

This study confirms Heyn's (1963) observation that there is intraspecific variation for *M. scutellata*. There are three morphological groups: 1) plants of low vigor with only one flower per inflorescence which bloom within 28 to 32 days after planting (35 accessions), 2) vigorous plants with three flowers per inflorescence which bloom 58 to 64 days after planting (21 accessions), and 3) plants which are intermediate between the previous two groups (18 accessions), and may have arisen from hybridization between them. There were seven accessions which contained mixtures of the vigorous and nonvigorous plants. The *M. rugosa* accessions are quite uniform morphologically, although three accessions have a more serrated leaflet and flower later than the other accessions.

Cytological observations of both *M. rugosa* and *M. scutellata* showed only counts of 2n = 30 in mitotic and meiotic figures (Fig. 1a to d). This is contrary to what is reported in the literature, as stated earlier. Diploid accessions were not found and meiosis was normal with 15 bivalents forming at metaphase I. This would suggest that both species are allotetraploids, as Heyn (1963) had presumed.

Medicago scutellata has two pairs of SAT-chromosomes (Fig. 1a) and *M. rugosa* has only one pair (Fig. 1c). One pair of satellites are separated from the main body of the chromosome by a relatively long distance. The satellites are large, especially in *M. rugosa* where the satellite is as large as the remainder of the chromosome. These observations were not made by earlier investigators and thus could explain the erroneous chromosome counts of 2n = 32, recorded previously.

There are several possible ways of deriving a somatic number of 2n = 30. One possibility is the hybridization of a 2n = 14 species and a 2n = 16 species followed by the polyploidization of the hybrid. Another possibility would be the loss of a pair of chromosomes from a 2n = 32 polyploid. The gain of a pair of chromosomes is unlikely because a 2n = 28 species has not been found in the genus *Medicago*.

Classen et al. (1982) have suggested, through the use of phenolic-taxometric studies, that the following species show a relationship to *M. scutellata*: *M. doliata* Carmian, *M. rigidula* (L.) All., *M. murex* Willd., *M. muricoleptis* Willd., and *M. rotata* Boiss. Two of the species are 2n = 14 (*M. murex* and *M. rigidula*) and the others are 2n = 16. In the same study, these same species plus *M. arabica* (L.) Bart., *M. blancheana* boiss., *M. coronata* (L.) Bart., *M. intertexta* (L.) Miller, *M. praecox* DC., and *M. polymorpha* L. have shown a relationship to *M. rugosa*. Again this list includes both 2n = 14 (*M. praecox* and *M. polymorpha*) and 2n = 16 species. This study suggests that the 2n = 30 species may have arisen through hybridization followed by polyploidization.

Further investigations into the origin of the 2n = 30 species may ultimately lead to the successful transfer of the glandular-haired trait from *M. scutellata*, *M. rugosa*, and other annual species to *M. sativa*.

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